

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>A01N 1/02, A61L 2/00, C12N 7/04</b>		A1	(11) International Publication Number: <b>WO 95/18529</b> (43) International Publication Date: <b>13 July 1995 (13.07.95)</b>
(21) International Application Number: <b>PCT/US95/00126</b> (22) International Filing Date: <b>4 January 1995 (04.01.95)</b>		(81) Designated States: CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 08/177,618                  4 January 1994 (04.01.94)                  US		Published <i>With international search report.</i>	
(71) Applicant: ORGANOGENESIS, INC. [US/US]; 150 Dan Road, Canton, MA 02021 (US).			
(72) Inventor: KEMP, Paul, D.; 7 Meadowcroft Road, Winchester, MA 01890 (US).			
(74) Agents: BAKER, Hollie, L. et al.; Hale and Dorr, 1455 Pennsylvania Avenue, N.W., Washington, DC 20004 (US).			

**(54) Title:** PERACETIC ACID STERILIZATION**(57) Abstract**

This invention is directed to the sterilization of collagen and collagenous tissue which is to be used for implantation, repair, or use in a mammalian host in the emerging field of tissue engineering. The sterilants are low concentration peracetic acid solutions in either neutral or high ionic strength that prevent or minimize the swelling of collagen or collagenous tissue so that the sterilized tissue retains its structural integrity and bioremodelable properties.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## PERACETIC ACID STERILIZATION

### 5 FIELD OF THE INVENTION

This invention is in the field of tissue engineering. The invention is directed to the sterilization of collagen and collagenous tissue which is to be used for implantation, repair, or use in a mammalian host. The sterilants are low or dilute concentrations of peracetic acid in either neutral or high ionic strength solutions that permit sterilization of the collagenous tissue with a minimal amount of swelling and dissolution so that the collagen retains its structural integrity and bioremodelable properties.

### BACKGROUND OF THE INVENTION

15 Tissue engineering is an emerging field combining both methods of the engineering and the principles of life sciences to understand the structural and functional relationships in normal and pathological mammalian tissues. The goal of tissue engineering is the development and ultimate application of biological substitutes to restore, maintain or improve tissue functions. Skalak, R. and Fox, C.F.,  
20 "Tissue Engineering," Alan R. Liss Inc. N.Y. (1988).

One major component of these biological substitutes can be collagen or collagenous tissue. While major strides have been made in this field, and are continuing to be made, there is a constant need for improvement of both the biological substitutes and the processes for making and using them. There currently exists a need  
25 for a sterilization method that maintains the biological and physical properties of collagen and collagenous tissues used in tissue engineering. The search for sterilants

to sterilize collagen and collagenous tissue has resulted in the use of a variety of different methods.

Physical sterilization methods have included heat, such as boiling, autoclaving, and microwaves. Heat, however, coagulates soft tissues. Additionally, heat at 5 temperatures above 60° to 65°C will denature collagen. Currently, gamma-irradiation is the preferred technique used to sterilize tissue, using between 0.5 and 2.5 megarads. A recent study has found, however, that collagen is damaged by gamma-irradiation at 1 megarad. Cheung *et al.*, "The Effect of Gamma-Irradiation on Collagen Molecules Isolated Alpha Chains and Crosslinked Native Fibers," *J. Biomedical Material Research*, 24:581-590 (1990). These lower dose ranges are used since irradiation doses in 10 excess of 2 megarads has a detrimental biological effect. Schnell *et al.*, "The Influence of Ionizing Radiation on Various Collagen-Containing Medical Products," *Radiosterilization of Medical Products*, Paper SM92, International Atomic Energy Agency, Vienna (1967).

Known chemical sterilization methods produce chemical reactions that not only 15 sterilize collagen, but also cross-link it. Collagen, cross-linked by chemical sterilization, results in a collagen tissue that is stiffer than uncross-linked collagen, remodels less well, and can evoke an immunological reaction. Thus, the chemical sterilants formaldehyde and glutaraldehyde cross-link collagen and reduce its capacity to 20 remodel after implantation. Kato *et al.*, *Journal Biol. Jt. Surgery*, 73:561 (1991).

Additionally, not all chemical sterilants are suitable for collagen sterilization. Ethyl and isopropyl alcohol are not sporicidal. Organic mercurials are toxic and have 25 an injurious effect on bone osteoinductive protein. Beta-propiolactone is only effective for surface sterilization and, because of its carcinogenicity, it has been removed from the market. Ethylene oxide has been used successfully for sterilization of bone, but can dissolve soft tissue.

All of the sterilization techniques mentioned above have some drawbacks or are only partially effective for the sterilization of collagen. The ideal sterilant must be able to sterilize without altering the essential physical and biological nature of the collagen.

Peracetic acid is a known germicidal sterilant. In medical applications, aqueous 5 or aqueous-ethanol peracetic acid solutions have been used typically to sterilize surfaces of instruments. Malchesky, P.S., "Peracetic Acid and Its Application to Medical Instrument Sterilization," *Artificial Organs*, 17:147-152 (1993). Sterilization of collagenous tissues by peracetic acid using these known techniques, however, is adversely affected by the swelling and dissolution of the collagen caused by the acidity 10 of the solution.

Thus, there continues to be a need for a sterilant that can effectively sterilize collagen intended for use as a biological substitute in tissue engineering applications that maintains the biological and physical properties of the collagen or collagenous material.

15

### SUMMARY OF THE INVENTION

The inventors have discovered that the use of low or dilute concentrations of peracetic acid in either neutral or high ionic strength solutions can prevent or minimize the swelling and dissolution of collagen and collagenous tissue so that the 20 tissue retains its structural integrity and bioremodelable properties. These peracetic acid solutions can be successfully used to sterilize biomedical products containing collagen or collagenous tissue that are to be used for implantation, repair, or use in tissue engineering without the attendant problems due to traditional sterilization methods.

## DETAILED DESCRIPTION OF THE INVENTION

The inventors have found that peracetic acid diluted in a neutral or high ionic strength solution is an effective sterilant for collagen and collagenous tissue, 5 preventing or minimizing the swelling and dissolution of the tissue. Peracetic acid is a strong oxidizing agent, sold commercially in approximately 35% concentration. A low concentration or dilute peracetic acid solution will typically contain less than 5% peracetic acid, preferably less than 1%, typically in a range between 0.1% to 0.5%, and more preferred less than 0.5%, typically in a range between 0.02% to 0.1%. The strength 10 of the peracetic acid in the dilute peracetic acid solution is not critical, but it is preferred to use a lower concentration of peracetic acid so that tissue is not damaged. The degree of the microbiological load, or bioburden, of the collagen or collagenous tissue may indicate that a higher concentration of peracetic acid is needed, and the solution can be adjusted accordingly.

15 The aqueous solution used to dilute the peracetic acid can be any solution that is compatible with collagen, for example, buffered or unbuffered water or saline, or solutions of sodium chloride. The preferred solution is either a buffered solution for ease in adjusting the pH of the dilute peracetic acid solution to neutral pH or a solution of 1M sodium chloride. Storage solutions for the collagenous bone grafts can 20 also be used to dilute the peracetic acid; these are typically phosphate buffered saline solutions.

In one embodiment of this invention, the pH of the low concentration, or dilute, peracetic acid solution is adjusted to neutralize the pH of the solution. Typically, the pH of the dilute peracetic acid solution is first measured with a pH meter 25 or with pH paper. Next, the solution is slowly neutralized by adding a base to the solution. Adjusting the pH is accomplished more easily by first dissolving the base in an aqueous solution. The pH is then adjusted until the pH of the solution is neutral.

As used herein, the term "neutral pH", is meant to cover a pH range from about 6.0 to about 8.0 pH. The preferred pH range is from about 7.0 to about 7.5 pH.

In another embodiment, the sterilizing solution is made from peracetic acid diluted in an aqueous solution with a high ionic strength, typically a high salt concentration. As used herein a "high salt concentration" can be any concentration of salt that is effective to prevent collagen from swelling at low pH. Preferably, the aqueous high salt concentration solution can be any concentration from 500mM to 3M, preferably from about 1M to about 2M. The salt that can be used in this invention can be sodium chloride, potassium chloride, calcium chloride, and the like. This dilute peracetic acid sterilizing solution with high salt concentration does not need to be pH adjusted.

The actual sterilization step is accomplished by soaking or washing the collagen or collagenous tissue with the dilute peracetic acid sterilization solution for a time and under conditions sufficient to achieve sterilization. The time for the sterilization will depend in part upon the size and type of the collagen or collagenous tissue to be sterilized, the manner of applying the sterilization solution, and the amount of bioburden. The sterilization time will typically be from about 5 minutes to about 30 hours.

The peracetic acid sterilization solution can be rinsed from the collagen or collagenous tissue, typically with the sterile water, saline, buffer, or storage media. Alternatively, the tissue graft can be placed directly into the sterile storage media without rinsing.

Since peracetic acid in dilute quantities degrades quickly, the peracetic acid sterilization solution should be used before it reduces. According to S. Block, *Disinfection, Sterilization, and Preservation*, 4th Edition, Lea & Febiger (1993), page 176, for example, a dilute peracetic acid solution of 1% loses half its strength through hydrolysis in 6 days.

Collagen and collagenous tissue that can be sterilized according to this invention can include autogenous, allogeneous, and xenogenous materials. Among these biomedical materials that can be sterilized are, but are not limited to, veins, tendons, dermis, heart valves, stomach smooth muscle tissue, and small intestine submucosa.

Collagenous tissue, as used herein, also includes collagen matrix structures and constructs. Since collagen is the basic building block that provides the structural framework for dermis, arteries, veins, tubular structures and most other human organs, the ability of the peracetic acid sterilant of this invention to sterilize collagenous biomaterials without damage is important.

Collagen tissue, matrix biomaterial and constructs, used as implants, in repair, or in a mammalian host, are capable of being remodeled into functional tissue by the host. Dense Fibrillar Collagen (DFC), as described in U.S. patent application serial no. 07/772,579, is a homogenous natural collagen that can be remodeled into living tissue and organ equivalents by the host. DFC can be produced in many forms, allowing for the development of many different collagen-based products. These biomedical products have broad applications in organ and tissue replacement therapy because they are designed to be similar to their counter parts in the human body and encourage natural healing.

Although the following invention is illustrated by the examples below for purposes of clarity and understanding, it is not intended to be limited to the examples.

### EXAMPLE 1

#### Preparation of Neutral Peracetic Acid Solution

The following example describes the preparation of the sterilant: neutral peracetic acid solution.

**Materials:**

5            Phosphate Buffered Saline (PBS) at 4°C  
          Peracetic Acid (35% concentration)  
          10N Sodium Hydroxide (NaOH)  
          Pasteur pipette and bulb  
          1 500 mL sterile Nalgene bottle  
          pH Meter

10          Phosphate buffered saline (PBS) solution was made from 0.144 grams potassium phosphate monobasic; 3.00 grams sodium phosphate dibasic (7H<sub>2</sub>O); 9.00 grams sodium chloride; and 1 liter purified water.

**Procedure:**

1.        349 mL PBS was added to a 500 mL sterile Nalgene bottle.
2.        1.4 mL of peracetic acid was added to the PBS.
3.        The bottle containing the peracetic acid solution was capped and shaken

15          gently.

4.        A stir bar was added to the bottle and the bottle was placed on a stir plate.
5.        The pH of the peracetic acid solution was measured with a pH meter and read between pH 4.0-5.0.

20          6.        With a Pasteur pipette, the solution was neutralized by adding 10N NaOH dropwise until the pH was between 7.0 and 7.2. The total volume of NaOH solution added was about 0.4-0.8 mL. As the pH reaction was slow, the NaOH solution was added slowly.

7.        The volume of the solution was increased with PBS to the 500 mL indicator to produce a final peracetic acid concentration of 0.1%.

**EXAMPLE 2****Preparation of a High Ionic Strength Peracetic Acid Solution**

The following example describes the preparation of the sterilant: dilute, high salt concentration, peracetic acid solution.

**5 Materials:**

Peracetic Acid (35% concentration)

Sodium Chloride (NaCl)

1 500 mL sterile Nalgene bottle

**Procedure:**

- 1 0      1. 29.22 grams of NaCl was dissolved to a volume of 500 mls with water.  
          2. 1.4 ml of 35% peracetic acid was added to make a 0.1% solution.

**EXAMPLE 3****Intestinal Collagen Material Graft Sterilization****1 5 Materials:**

Three, one-foot (1') sections of porcine intestinal collagen material  
Freshly prepared, sterile peracetic acid solutions as prepared  
in Example 1 and Example 2

Sterile Phosphate Buffered Saline (PBS)

**2 0 Procedure:**

1. The small intestine of a pig was harvested and then, when trimmed into a sheet, mechanically stripped and cleaned so that the tunica submucosa was delaminated and separated. The submucosa was separated from the small intestine by mechanically squeezing the raw material between opposing rollers. The tunica submucosa of the small intestine is harder and stiffer than the surrounding tissue, and the rollers squeeze the softer components from the submucosa.

2. Three, one-foot samples of cleaned porcine intestinal collagen material were immersed in 100 ml of neutral, 0.1% peracetic acid solution, prepared as described in Example 1, for 16, 40, or 62 hours (three samples for each time point.)

3. One-inch samples were taken from each one-foot sample of intestinal collagen material after incubation for the appropriate time in neutral, 0.1% peracetic acid. These samples were aseptically transferred to either sterile tryptic soy broth (TSB) or thioglycollate media (Thio) and incubated for fourteen days at 37°C or 30°C, respectively. One-inch samples were also taken from untreated intestinal collagen material and similarly incubated for fourteen days in TSB or Thio to serve as controls.

5 Results:

All experimental peracetic acid treated graft samples, whether incubated for 16, 40, or 62 hours, were sterile after fourteen days in either TSB or Thio. Sections 10 from untreated controls were all contaminated with *Klebsiella pneumoniae* and *Escherichia coli* as seen after 14 days in either TSB or Thio.

#### Example 4

15 Bacterial Challenge

Materials:

Porcine intestinal collagen material

*Bacillus subtilis* spores

Neutral peracetic acid solution as described in Example 1

20 Liquid Thioglycollate Broth (Thio)

Tryptic Soy Broth (TSB)

Procedure:

1. A sample of intestinal collagen material, as described in Example 3, was inoculated with 2 million *B. subtilis* spores per foot length of material.

25 2. One-foot samples of inoculated material were immersed in 150 ml of neutral 0.1% peracetic acid solution and incubated for 8 hours or 16 hours (6 samples for each time point).

30 3. The one-inch samples of neutral peracetic acid-treated intestinal collagen material and controls (inoculated with *B. subtilis* but not peracetic acid treated) were aseptically transferred to either TSB or Thio and incubated for 14 days (Incubated at 37°C or 30°C, respectively).

**Results:**

All peracetic acid treated samples of intestinal collagen material were sterile. Control samples, spiked with *B. subtilis* spores, gave positive growth in TSB after 1 day incubation and in Thio after 3 days incubation.

5

**Example 5****Swelling of Intestinal Collagen Material  
in Peracetic Acid Solutions****10 Materials:**

- Porcine intestinal collagen material
  - 0.1% peracetic acid solution in water (pH 3.5)
  - 0.1% peracetic acid solution in phosphate buffered saline (pH 6.1)
  - 0.1% peracetic acid in phosphate buffered saline, neutralized to pH 7.2
- 15 as described in Example 1.
- 0.1% peracetic acid solution in 1M sodium chloride as described in Example 2 (pH 3.3)

**Procedure:**

Sections of intestinal collagen material (approximately 3 inches long) were 20 prepared as described in Example 3 and blotted dry, weighed and placed in 50 mls of one of the above dilute peracetic acid solutions (the experiment was performed in triplicate). After 19 hours incubation at room temperature, the samples were transferred to fresh solution and left for a further 28 hours. Samples were then removed, blotted briefly to remove surface liquid and re-weighed.

**Results:**

	Solution	Initial weight Average (grams)	Final Weight Average (grams)	Swelling Average (%)
5	0.1% PA (in water) pH 3.5	3.47 ± 0.3	5.07 ± 0.5	46
10	0.1% PA (in PBS) pH 6.1	2.97 ± 0.4	1.93 ± 0.1	-35
15	0.1% PA (in PBS) pH 7.2	3.43 ± 0.2	3.03 ± 0.3	-11.65
20	0.1% PA (in 1M NaCl)	3.03 ± 0.5	2.17 ± 0.6	-29

The sample of intestinal collagen material exposed to 0.1% PA in water swelled 46% over its pre-incubation weight. The other samples exposed to peracetic acid either at pH 6.1 or 7.2 or in 1M NaCl actually lost water compared to initial weight.

**Example 6****Swelling of DFC in Peracetic Acid Solutions****Materials:**

- Dense Fibrillar Collagen (DFC) Sheet produced as described in Application Serial No. 07/772,579
- 0.1% peracetic acid solution in water (pH 3.5)
- 0.1% peracetic acid solution in phosphate buffered saline (pH 6.1)
- 0.1% peracetic acid in phosphate buffered saline, neutralized to pH 7.2 as described in Example 1
- 0.1% peracetic acid solution in 1M sodium chloride as described in Example 2 (pH 3.3)

**Method:**

Sections of dry DFC sheet (stored dry for more than 6 months) were weighed and placed in 50 mls of one of the above solutions (the experiment was performed in duplicate). After 19 hours incubation at room temperature, the samples were removed, blotted briefly to remove surface liquid and re-weighed.

5 Results:

	Solution	Initial (dry) Weight Average (grams)	Final Weight Average (grams)	Swelling Average (%)
10	0.1% PA (in water) pH 3.5	0.12 ± 0.03	2.05 ± 0.35	1682
15	0.1% PA (in PBS) pH 6.1	0.2 ± 0.02	1.32 ± 0.1	574
20	0.1% PA (in PBS) pH 7.2	0.2 ± 0.02	1.18 ± 0.12	502
25	0.1% PA in (in 1M NaCl)	0.14 ± 0	0.47 ± 0.03	214
	PBS Control (No PA)	0.15 ± 0.01	1.2 ± 0.1	700

The DFC sample in 0.1% peracetic acid in water swelled more than the other samples. This sample did not dissolve due to covalent crosslink formation which occurs during storage of dry collagen materials. The samples exposed to peracetic acid either at pH 6.1 or 7.2 or in 1M NaCl swelled much less than the sample in 0.1% peracetic acid in water.

### Example 7

#### 5                   Shrinkage Temperature of DFC Threads Exposed to Various Peracetic Acid Solutions

##### Methods:

###### Thread Production:

Dense Fibrillar Collagen (DFC) thread production is described in U.S. Serial No. 07/772,529, incorporated herein by reference. As used for this experiment, a 140 ml syringe containing a 5.0 mg/ml collagen solution in 8.8 mM acetic acid was loaded in a syringe pump (Harvard Apparatus, South Natick, MA) set to infuse at 2.50 ml/min. Silicone tubing (1/8-inch ID) connected the syringe to an 18-gauge blunt stainless steel needle immersed in one end of an 18-foot long, 2-inch diameter PVC trough containing 5L of 20% polyethylene glycol (PEG), MW 8000 (Spectrum Chemicals, New Brunswick, NJ), in 94 mM sodium phosphate dibasic and 24 mM sodium phosphate monobasic at pH 7.55. A peristaltic pump was used to recirculate the PEG solution such that the fluid velocity at the needle tip was about 4 cm/sec. The circulating PEG solution drew the collagen away from the needle orifice, thus forming a continuous filament. The collagen gelled on contact with the neutral pH solution, and the nascent thread began to dehydrate due to the osmotic pressure gradient formed between the collagen and the PEG solution. The coagulation trough was configured such that the residence time of the thread in the bath was approximately 4 minutes. As the thread accumulated, it was manually transferred to a 6 foot long trough filled with 5.5 mM sodium phosphate dibasic, 0.5 mM potassium phosphate monobasic and 75 mM NaCl at pH 7.10, and remained there for 5 to 10 minutes.

The thread was then partially dehydrated by passing it through a 2 foot long trough containing 70% isopropanol, and dried under tension by drawing it over a series of Teflon pulleys inside a cabinet heated with air blowers. To prevent the

collagen from being heat denatured, the thread was kept in motion, and was always at least 15 cm from the blowers. Finally, the thread was spooled onto a level winding device. The tension on the thread during the drying was such that its length doubled before it emerged dry from the cabinet. The total residence time in  
5 the cabinet was approximately 3 minutes.

Once the filament was threaded through the entire system, it could be produced continuously; the operator needed only to keep the coagulation and rinsing reservoirs filled with enough slack to permit reeling.

Shrinkage Temperature Measurement:

10 Shrinkage temperature, a measure of the stability of the collagen triple helix, was measured by immersing a 5 to 7 cm loop of thread loaded with 2.5 g in 1.0 mM potassium phosphate monobasic, 11 mM sodium phosphate dibasic, and 150 mM NaCl at pH 7.30, and heating at 1°C per minute until shrinkage occurred. The temperature at which the sample shrank by at least 10% was recorded as the  
15 shrinkage temperature.

Peracetic Acid Treatment:

Lengths of collagen thread were exposed to the following solutions for 20 minutes:

- 1) 0.1% peracetic acid solution in water (pH 3.5);  
20 2) 0.1% peracetic acid solution in phosphate buffered saline (pH 6.1);  
3) 0.1% peracetic acid in phosphate buffered saline, neutralized to pH 7.2 as described in Example 1;  
4) 0.1% peracetic acid solution in 1M sodium chloride as described in Example 2 (pH 3.3).

25

**Results:**

Solution	Initial Shrinkage Temperature (°C)	Final Shrinkage Temperature (°C)
5      0.1% PA (in water) pH 3.5	47.5 ± 1.0	Dissolved
10     0.1% PA (in PBS) pH 6.1	47.5 ± 1.0	45.5 ± 1.0
15     0.1% PA (in PBS) pH 7.2	47.5 ± 1.0	47.5 ± 0.5
20     0.1% PA (in 1M NaCl)	46.5 ± 0.5	47.0 ± 1.0

The shrinkage temperature was not affected by treatment with peracetic acid. The collagen threads dissolved in 0.1% peracetic acid in water, but remained intact in the other solutions.

25

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one skilled in the art that certain changes and modifications may be practiced within the scope of the appended claims.

**CLAIMS:**

1. A method for sterilizing collagen or collagenous tissue comprising contacting said collagen or collagenous tissue with a low concentration peracetic acid solution that sterilizes said collagen or collagenous tissue with no or a minimal amount of swelling and dissolution wherein said sterilized collagen or collagenous tissue retains its structural integrity and bioremodelable properties.

2. A method for sterilizing collagen or collagenous tissue that is to be used in a mammalian host comprising:

(a) contacting said collagen or collagenous tissue with a low concentration peracetic acid solution for a time sufficient for sterilization, wherein said solution prevents or minimizes the swelling of said collagen or collagenous tissue and wherein said sterilized collagen or collagenous tissue retains its structural integrity and bioremodelable properties; and

(b) using said sterilized collagen or collagenous tissue in a mammalian host.

3. The method as in either claim 1 or claim 2, wherein said low concentration peracetic acid has a peracetic acid concentration of less than 5% and has a neutralized pH of from about 6.0 to about 8.0 pH.

4. The method of claim 3 wherein said neutralized pH is from about 7.0 to about 7.5 pH.

5. The method of claim 3 wherein said peracetic acid concentration is in the range of 0.02% to 5%.

6. The method of claim 5 wherein said peracetic acid concentration is in the range of 0.02% to 1.0%.

7. The method as in either of claim 1 or claim 2, wherein said low concentration peracetic acid has a peracetic acid concentration of less than 5% and has a high salt concentration.

8. The method of claim 7 wherein said high salt concentration is from about 500mM to about 3M.

9. The method of claim 8 wherein said high salt concentration is from about 1M to about 2M.

5 10. The method of claim 7 wherein said peracetic acid concentration is in the range of 0.02% to 5%.

11. The method of claim 10 wherein said peracetic acid concentration is in the range of 0.02% to 1.0%.

12. A sterilant for collagen or collagenous tissue for use in tissue engineering  
10 consisting essentially of a low concentration peracetic acid solution that prevents or minimizes the swelling of said collagen or collagenous tissue and wherein said sterilized collagen or collagenous tissue retains its structural integrity and bioremodelable properties.

13. A sterilant for collagen or collagenous tissue for use in tissue engineering  
15 consisting essentially of a low concentration peracetic acid solution of less than 5% in a neutralized pH solution, said neutralized pH ranging from about 6.0 to about 8.0 pH.

14. A sterilant for collagen or collagenous tissue for use in tissue engineering consisting essentially of a low concentration peracetic acid solution in a high salt concentration solution.

20 15. The sterilant of any of claims 12-14 wherein said peracetic acid is less than 5% concentration.

16. The sterilant of claim 14 wherein said peracetic acid concentration is in the range of 0.02% to 5%.

25 17. The sterilant of claim 16 wherein said peracetic acid concentration is in the range of 0.02% to 1.0%.

18. The sterilant of claim 14 wherein said high salt concentration is from about 500mM to about 3M.

19. The sterilant of claim 18 wherein said high salt concentration is from about 1M to about 2M.

20. A method for sterilizing collagen or collagenous tissue comprising contacting said collagen or collagenous tissue with a low concentration peracetic acid 5 solution that sterilizes said collagen or collagenous tissue with no or a minimal amount of swelling and dissolution wherein said solution has a peracetic acid concentration in the range of 0.02% to 1.0% and has a neutralized pH of from about 6.0 to about 8.0 pH.

10 21. The method of claim 20 wherein said solution has a peracetic acid concentration of about 0.1% and has a neutralized pH of from about 6.0 to about 8.0 pH.

15 22. A method for sterilizing collagen or collagenous tissue comprising contacting said collagen or collagenous tissue with a low concentration peracetic acid solution that sterilizes said collagen or collagenous tissue with no or a minimal amount of swelling and dissolution wherein said solution has a peracetic acid concentration in the range of 0.02% to 1.0% and has a high salt concentration from about 1M to about 2M.

20 23. The method of claim 22 wherein said solution has a peracetic acid concentration of about 0.1% and has a high salt concentration from about 1M to about 2M.

## INTERNATIONAL SEARCH REPORT

Int'l application No.

PCT/US95/00126

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A01N 1/02, A61L 2/00, C12N 7/04  
 US CL : 435/1, 236; 422/28

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/1, 236; 422/28

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CAS ONLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Please See Continuation of Second Sheet.	

 Further documents are listed in the continuation of Box C.

See patent family annex.

- \* Special categories of cited documents:
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "E" earlier document published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "Z" document member of the same patent family

Date of the actual completion of the international search

16 FEBRUARY 1995

Date of mailing of the international search report

31 MAR 1995

Name and mailing address of the ISA/US  
 Commissioner of Patents and Trademarks  
 Box PCT  
 Washington, D.C. 20231  
 Facsimile No. (703) 305-3230

Authorized officer *Sandra Saucier*  
 SANDRA SAUCIER  
 Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

Int'l. application No.

PCT/US95/00126

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	Z. exp. Chir. Transplant. kunstl. Organe, Volume 22, issued 1989, von Versen et al., "The peracetic acid/low pressure cold sterilization, pages 18-21, see entire article.	1 -- 2-11, 20-23
X — Y	Khirurgiya (Sofia), Volume 29, Number 4, issued 1976, Wenzel et al., "Comparative study of the cold sterilization of transplant tissue", pages 303-307, see abstract.	1 ----- 2-11, 20-23
X — Y	Z. exp. Chir. Transplant. kunstl. Organe, Volume 17, Number 5, issued 1984, Starke et al., "Experimental studies of the sterilization of transplantation material with peracetic acid", pages 254-258, see abstract.	1 ----- 2-11, 20-23
X —, P Y	US, A, 5,298,222 (O'LEARY) 29 March 1994, see column 3 line 68 and column 4, line 48.	1 --- 2-11, 20-23
X — Y	US,A,4,900,721 (BANSEMIR ET AL.) 13 February 1990, see claims 7, 15 and abstract.	1, 7, 10-12, 14-17 ----- 2-6, 8, 9, 18, 19 20-23
X — Y	US,A, 5,116,575 (BADERTSCHER ET AL.) 26 May 1992, see column 6, lines 26-60 and Table 1.	1, 3-7, 10- 17 ----- 2, 8, 9, 18-23
X — Y	Nature New Biology, Volume 240, issued 08 November 1972, Schweizer et al., "Peracetic Acid as Sterilizing Agent for Culture Serum", pages 61-62, see entire article.	12-17 ----- 18, 19
X — Y	US,A, 5,120,656 (O'LEARY) 09 June 1992, see column 2, lines 45-54 and column 3, line 63.	12-14 ----- 15-19